

Serial No. 09/908,950 filed 7/19/01
Response of 12/27/05 to Office Action of 6/27/05

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Previously Presented) A method for determining the presence of a specific nucleotide sequence in an RNA reagent of a target sample, said method comprising the steps of:
 - a) incubating a mixture comprising:
 - (i) a first component including an RNA reagent extracted from a target sample, said RNA reagent having a target nucleotide sequence and a capture sequence, and
 - (ii) a second component comprising a capture reagent comprising a label capable of emitting a detectable signal and comprising a nucleotide sequence complementary to the capture sequence of the RNA reagent of the first component,at a first temperature to induce the capture sequence of the RNA reagent of the first component to bind to the complementary nucleotide sequence of the capture reagent of the second component, and thereby form a pre-hybridized RNA-capture reagent complex comprising the target nucleotide sequence;
 - b) contacting the pre-hybridized RNA-capture reagent complex with a microarray having thereon a plurality of features each comprising a particular probe nucleotide sequence; and
 - c) incubating the pre-hybridized RNA-capture reagent complex on the microarray at a second temperature to hybridize the target nucleotide sequence of the pre-hybridized RNA-capture reagent complex to the complementary probe nucleotide sequence contained within the feature,

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wherein the presence of such hybridization results in a detectable hybridization pattern for subsequent analysis.

2. (Original) The method of claim 1 wherein the capture reagent is selected from the group consisting of dendrimers, carbohydrates, proteins, and nucleic acids.
3. (Original) The method of claim 2 wherein the capture reagent is a dendrimer.
4. (Original) The method of claim 1 further comprising passing a base solution over the microarray to separate and purge the hybridized RNA reagent from the probe nucleotide sequence for enabling reuse of the microarray.
5. (Original) The method of claim 4 wherein the base solution is passed over the microarray at a temperature of from about 50° to 60° C.
6. (Original) The method of claim 4 wherein the base solution is 0.05 M sodium hydroxide.
7. (Original) The method of claim 1 wherein the capture sequence of the RNA reagent is a single-stranded oligonucleotide consisting of at least one adenine base.
8. (Original) The method of claim 7 wherein the nucleotide sequence complementary to the capture sequence is a single-stranded oligonucleotide consisting of at least one thymine base.
9. (Original) The method of claim 1 wherein the RNA reagent and the capture reagent are incubated at the first temperature of from about 45° to 60° C.
10. (Original) The method of claim 9 wherein the RNA reagent and the capture reagent are incubated for a sufficient time ranging from about 15 minutes to 24 hours.
11. (Currently Amended) The method of claim 1 wherein the pre-hybridized RNA~~z~~-capture reagent

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complex is incubated on the microarray at the second temperature of from about 45° to 65 °C.

12. (Currently Amended) The method of claim 11 wherein the pre-hybridized RNA₊-capture reagent complex are incubated on the microarray for the sufficient time ranging from about 15 minutes to 24 hours.

13. (Original) The method of claim 11 wherein the probe nucleotide sequences of the microarray comprises cDNA.

14. (Original) The method of claim 11 wherein the second temperature is from about 60° to 65 °C.

15. (Original) The method of claim 11 wherein the probe nucleotide sequences of the microarray comprises oligonucleotides.

16. (Original) The method of claim 14 wherein the second temperature is from about 45° to 55°C.

17. (Currently Amended) The method of claim 1, ~~after incubating the pre-hybridized RNA/recapture reagent complex on the microarray~~, further comprising washing any free unhybridized RNA₊-capture reagent complex from the microarray after incubating the pre-hybridized RNA-capture reagent complex on the microarray.

18. (Currently Amended) The method of claim 1, ~~after incubation of the first and second components~~, further comprising adding blocking nucleic acids to the mixture of the first and second components after incubation of the first and second components.

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19. (Previously Presented) A method for determining the presence of a specific nucleotide sequence in an RNA reagent of a target sample, said method comprising the steps of:

- a) contacting a first component including an RNA reagent extracted from a target sample, said RNA reagent having a target nucleotide sequence and a capture sequence with a microarray having thereon a plurality of features each comprising a particular probe nucleotide sequence;
- b) incubating the RNA reagent and the complementary probe nucleotide sequences on the microarray at a first temperature to hybridize the target nucleotide sequence of the RNA reagent to the complementary probe nucleotide sequence contained within the feature;
- c) contacting a second component comprising a capture reagent comprising a label capable of emitting a detectable signal and comprising a nucleotide sequence complementary to the capture sequence of the RNA reagent of the first component; and
- d) incubating the capture reagent and the capture sequence of the RNA reagent at a second temperature to induce the capture sequence of the RNA reagent of the first component to hybridize to the complementary nucleotide sequence of the capture reagent of the second component, wherein the presence of the hybridization results in a detectable hybridization pattern for subsequent analysis.

20. (Original) The method of claim 19 wherein the capture reagent is selected from the group consisting of dendrimers, carbohydrates, proteins, and nucleic acids.

21. (Original) The method of claim 20 wherein the capture reagent is a dendrimer.

22. (Original) The method of claim 19 further comprising passing a base solution over the

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microarray to separate and purge the hybridized RNA reagent from the probe nucleotide sequence for enabling reuse of the microarray.

23. (Original) The method of claim 22 wherein the base solution is passed over the microarray at a temperature of from about 50° to 60° C.
24. (Original) The method of claim 22 wherein the base solution is 0.05 M sodium hydroxide.
25. (Original) The method of claim 19 wherein the capture sequence of the RNA reagent is a single-stranded oligonucleotide consisting of at least one adenine base.
26. (Original) The method of claim 25 wherein the nucleotide sequence complementary to the capture sequence is a single-stranded oligonucleotide consisting of at least one thymine base.
27. (Original) The method of claim 19 wherein the RNA reagent and the complementary probe nucleotide sequences on the microarray are incubated at the first temperature of from about 45° to 65° C.
28. (Original) The method of claim 27 wherein the RNA reagent and the complementary probe nucleotide sequences on the microarray are incubated for a sufficient time ranging from about 15 minutes to 24 hours.
29. (Original) The method of claim 27 wherein the probe nucleotide sequences of the microarray comprises cDNA.
30. (Original) The method of claim 29 wherein the first temperature is from about 60° to 65° C.
31. (Original) The method of claim 27 wherein the probe nucleotide sequences of the microarray comprises oligonucleotides.
32. (Original) The method of claim 31 wherein the second temperature is from about 45° to 55° C.

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33. (Currently Amended) The method of claim 19 wherein the capture reagent and the capture sequence of the RNA reagent are incubated at a second temperature ranging from about 45° to 60°C.

34. (Original) The method of claim 33 wherein the capture reagent and the capture sequence of the RNA reagent are incubated for the sufficient time ranging from about 15 minutes to 24 hours.

35. (Currently Amended) The method of claim 19, ~~after incubating the capture sequence of the RNA reagent and the capture reagent on the microarray~~, further comprising adding blocking nucleic acids to the microarray after incubating the capture sequence of the RNA reagent and the capture reagent on the microarray.

36. (Currently Amended) The method of claim 19, ~~after the incubating the RNA reagent and the complementary nucleotide probes on the microarray step~~, further comprising washing the microarray with a buffer solution to remove excess unhybridized RNA reagent after the incubating the RNA reagent and the complementary nucleotide probes on the microarray step.

37. (Original) The method of claim 1 wherein the capture sequence of the RNA reagent comprises at least one locked nucleic acid nucleotide.

38. (Original) The method of claim 19 wherein the capture sequence of the RNA reagent comprises at least one locked nucleic acid nucleotide.

39. (Previously Presented) The method of claim 1, wherein said probe nucleotide sequence is DNA.

40. (Previously Presented) The method of claim 1, wherein said probe nucleotide sequence is RNA.

41. (Previously Presented) The method of claim 19, wherein said probe nucleotide sequence is DNA.

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42. (Previously Presented) The method of claim 19, wherein said probe nucleotide sequence is RNA.
43. (Previously Presented) The method of claim 1, wherein said second component comprises a capture reagent having at least one first arm comprising said label and at least one second arm having comprising said nucleotide sequence complementary to said capture sequence of the RNA reagent.
44. (Previously Presented) The method of claim 43, wherein said second component is a dendrimer.
45. (Previously Presented) The method of claim 19, wherein said second component comprises a capture reagent having at least one first arm comprising said label and at least one second arm having comprising said nucleotide sequence complementary to said capture sequence of the RNA reagent.
46. (Previously Presented) The method of claim 45, wherein said second component is a dendrimer.
47. (Previously Presented) A method comprising the steps of:
- (a) using an array of probe nucleotide sequences;
 - (b) using a first component comprising RNA reagent, said RNA reagent having a target nucleotide sequence and a capture sequence;
 - (c) using a second component comprising a complement, said complement being a complementary nucleotide sequence to said capture sequence of said RNA reagent;
 - (d) contacting said RNA reagent with both said array and a second component in any order;
 - (e) wherein said RNA is contacted with said array to allow said target nucleotide sequence

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- of said RNA reagent to bind to any of said probe nucleotide sequences on said array that comprise DNA or RNA complementary to said target nucleotide sequence;
- (f) wherein said RNA reagent is contacted with said second component to allow said complement to bind to said capture sequence of said RNA reagent;
- (g) and wherein said second component produces a detectable hybridization pattern on said array.
48. (Previously Presented) The method of claim 47, wherein said second component comprises a dendrimer.
49. (Previously Presented) The method of claim 47, wherein said second component comprises at least one molecule selected from the group consisting of dendrimers, carbohydrates, proteins, and nucleic acids.
50. (Previously Presented) The method of claim 47, wherein said capture sequence comprises at least one adenine base.
51. (Previously Presented) The method of claim 47, wherein said capture sequence comprises a poly A tail.
52. (Previously Presented) A composition, said composition comprising:
- (a) an array of probe nucleotide sequences;
- (b) said array further comprising a first component comprising RNA reagent, said RNA reagent having a target nucleotide sequence and a capture sequence, said target nucleotide sequence of said RNA reagent being bound to one of said probe nucleotide

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sequences on said array, wherein said target sequence of said RNA reagent is bound to a probe nucleotide sequence of DNA or RNA;

- (c) said composition further comprising a second component, said second component comprising a complementary nucleotide sequence to said capture sequence of said RNA reagent, said complementary nucleotide sequence being bound to said capture sequence;
 - (d) and wherein said second component further comprises a label.
53. (Currently Amended) The method composition of claim 52, wherein said second component comprises a dendrimer.
54. (Currently Amended) The method composition of claim 52, wherein said second component comprises at least one molecule selected from the group consisting of dendrimers, carbohydrates, proteins, and nucleic acids.
55. (Currently Amended) The method composition of claim 52, wherein said capture sequence comprises at least one adenine base.
56. (Currently Amended) The method composition of claim 52, wherein said capture sequence comprises a poly A tail.